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Serial Number: 09/320,100 Results Format Preferred (circle): PAPER DISK E-MAIL

Title of Invention Separation of polypeptide monomers

Inventors (please provide full names): Deborah Ann Ansaldi

Earliest Priority Date: 06/01/1998

Point of Contact:
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CM1 1D16 Tel: 308-4258

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Keywords (include any known synonyms registry numbers, explanation of abbreviations):
macromonomers oligomers macromers
• MONOMERS, DIMERS, MULTIMERS ANTIBODIES,
blood proteins milk proteins SERUM ALBUMIN, Ab, anti-IgE, anti-IgG,
albumin anti-Her2, anti-CD11a, anti-CD18, anti-CD20,
anti-VEGF, or anti-IgE
base exchange ion exchange
• cation-exchange, anion-exchange
• separating / purifying • step-wise gradient
linear gradient
• elution salt - Sodium salt, Sodium chloride

Search Topic:

Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

a method of separating (purifying) monomers from dimers and/or multimers of the monomer using cation-exchange at buffer pH of 4-7 or anion-exchange at buffer pH of 6-9, and eluting the mixture at a gradient of about 0-1M of an elution salt. Monomer purity is >99.5% and yield is >90%. Thanks.

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=> s (macromonomer! or monomer! or macromer! or dimer! or multimer! or
oligomer? or antibod? or serum albumin or ab or antibody or anti ige or anti
igg or blood protein or milk protein or albumin or anti her2 or anti cdiia or
cd18 or anti cd20 or anti vegf or ige)
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L1       855201 FILE MEDLINE
L2       794126 FILE CAPLUS
L3       614980 FILE BIOSIS
L4       539657 FILE EMBASE
L5       128242 FILE WPIDS

TOTAL FOR ALL FILES
L6      2932206 (MACROMONOMER! OR MONOMER! OR MACROMER! OR DIMER! OR MULTIMER!
                OR OLIGOMER? OR ANTIBOD? OR SERUM ALBUMIN OR AB OR ANTIBODY OR
                ANTI IGE OR ANTI IGG OR BLOOD PROTEIN OR MILK PROTEIN OR
ALBUMIN
                 OR ANTI HER2 OR ANTI CDIIA OR CD18 OR ANTI CD20 OR ANTI VEGF
                OR IGE)

=> s l6 and ((cation or anion or base or ion)(w)exchange?)

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L9          4495 FILE BIOSIS
L10         3582 FILE EMBASE
L11         2007 FILE WPIDS

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L15         3288 FILE BIOSIS
L16         2548 FILE EMBASE
L17          840 FILE WPIDS

TOTAL FOR ALL FILES
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=> s l18 and (elut? salt or sodium salt or na salt or sodium chloride or nacl)

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L20          344 FILE CAPLUS
L21          137 FILE BIOSIS
L22          117 FILE EMBASE
L23           72 FILE WPIDS

TOTAL FOR ALL FILES
L24          863 L18 AND (ELUT? SALT OR SODIUM SALT OR NA SALT OR SODIUM
CHLORIDE
                 OR NACL)

=> s (step wise or linear)(w)gradient? and l24

L25            8 FILE MEDLINE
L26           16 FILE CAPLUS
L27           13 FILE BIOSIS
L28            8 FILE EMBASE
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L29 4 FILE WPIDS

TOTAL FOR ALL FILES
L30 49 (STEP WISE OR LINEAR)(W) GRADIENT? AND L24

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PROCESSING COMPLETED FOR L30
L31 22 DUP REM L30 (27 DUPLICATES REMOVED)

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L31 ANSWER 1 OF 22 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-452385 [39] WPIDS
AB WO 200039280 A UPAB: 20001106
NOVELTY - Obtaining a highly **purified** mixture of type I interferon (INF), of more than one subtype, from leukocytes where the mixture has a purity of at least 95 % and contains no more than about 35 % by weight INF alpha -2 and INF alpha -8 subtypes comprises sequential **purification** from cultured and stimulated leukocytes.

DETAILED DESCRIPTION - Obtaining a highly **purified** mixture of type I (INF), of more than one subtype, from leukocytes where the mixture has a purity of at least 95 % and contains no more than about 35 % by weight INF alpha -2 and INF alpha -8 subtypes comprises:

(a) culturing leukocytes;

(b) stimulating the leukocytes to produce a crude interferon;

(c) concentrating the crude interferon to remove low molecular weight contaminants;

(d) removing a substantial amount of **serum albumin** and other contaminants from the concentrated crude interferon to produce a partially **purified** interferon mixture containing a plurality of subtypes;

(e) removing substantially all remaining **serum albumin** and other contaminants from the partially **purified** interferon mixture to generate an interferon mixture having a purity of 50 - 80 %; and

(f) **purifying** the mixture of (f) to produce a highly **purified** mixture of type I interferon with a purity of 95 % and containing no more than about 35 % by weight IFN alpha -2 and IFN alpha -8 subtypes.

INDEPENDENT CLAIMS are also included for the following:

(1) transporting viable blood cells comprising maintaining the temperature of the blood cells at 22 plus or minus 3 deg. C in an isotonic medium and maximizing the degree of oxygenation of the blood cells;

(2) apparatus for transporting the blood cells comprising an insulating container with a lid, which has means for hanging bags containing blood cells **separate** from one another to allow the free flow of oxygen between the bags;

(3) maximizing interferon production from a buffy coat comprising:

(a) collecting a buffy coat, maintaining the temperature at 22 plus or minus 3 deg. C with sufficient oxygenation to retain cell viability;

(b) **purifying** the buffy coat by density gradient centrifugation;

(c) collecting a preparation of at least 10 % monocytes with less than 10 % granulocytes; and

(d) culturing the preparation to produce interferon;

(4) isolating peripheral blood mononuclear cells comprising:

(a) collecting a buffy coat at 22 plus or minus 3 deg. C;

(b) washing the buffy coat with isotonic PBS at 22 plus or minus 3 deg. C until the buffy coat is isotonic;

(c) preparing a density gradient comprising 1 volume of isotonic Percoll(TM) with a density of 1.07 g/ml overlaid on 4 volumes LS(TM) with a density of 1.077 - 1.08 g/ml with a slightly mixed interface;

(d) overlaying the isotonic buffy coat onto the density gradient and centrifuging at 22 plus or minus 3 deg. C until a peripheral blood mononuclear cell layer is formed; and

(e) collection of the peripheral blood mononuclear cells;

(5) producing interferon from peripheral blood mononuclear cells comprising:

(a) culturing peripheral blood mononuclear cells in a serum-free culture medium with interferon primer at 37 deg. C for 2 hours;

(b) incubating the peripheral mononuclear cells with inducing virus for about 2 hours;

(c) reducing the temperature of the peripheral blood mononuclear cells to 28 deg. C for 14 hours; and

(d) harvesting the interferon-containing serum-free culture medium;

(6) a natural mixture of type I interferon which is at least 95 % pure comprising subtypes IFN- alpha 1a, IFN- alpha 1new, IFN- alpha 2a, IFN- alpha 2b, IFN- alpha 2c, IFN- alpha 5, IFN- alpha 5LG, IFN- alpha 7, IFN- alpha 8a, IFN- alpha 8c, IFN- alpha 10a, IFN- alpha 14a, IFN- alpha 14b, IFN- alpha 14c, IFN- alpha 14LG, IFN- alpha 21a, IFN- alpha 21b, IFN- alpha 21c, IFN- omega and IFN- omega LG;

(7) **purifying** interferon away from human **serum albumin** comprising:

(a) applying a sample of crude interferon to a size exclusion column under dissociating, non-denaturing conditions, where the column is capable of separating proteins of a size range 3 - 70 kDa; and

(b) washing the column with a low salt buffer at neutral pH until the human serum albumin has been removed from the column and the collecting the interferon from the column; and

(8) purifying a natural mixture of type I interferon comprising;

(a) applying a sample of 40 - 60 % type I interferon to a quaternary amino anion exchange column in a medium salt buffer at about neutral pH;

(b) washing the column with medium salt buffer at a neutral pH; and

(c) eluting the interferon with an increasing step or linear gradient of a high salt buffer at neutral pH, where the eluted interferon comprises a neutral mixture of type I interferons having at least 9 subtypes and at least 16 molecular species.

USE - The natural mixture of type I IFN can be used to treat

interferon responsive diseases e.g. hepatitis A infection, hepatitis B infection, hepatitis C infection, HIV infection, herpes zoster virus infection, influenza infection, common cold infections, hemorrhagic fever infections, genital warts, bacterial infections, chlamydia infection, Behcet's disease, Churg-Strauss syndrome, leukemia, T-cell leukemia, chronic myeloid leukemia, melanoma, myofibromatosis, T-cell lymphoma, basal cell carcinomas, squamous cell carcinomas, renal cell carcinoma, colorectal carcinoma, non-small cell lung cancer, cervical cancer, breast cancer, gastrointestinal malignancies, actinic keratoses, macular degeneration, autoimmune disorders, diabetes, psoriasis, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus (claimed).

ADVANTAGE - The method provides an inexpensive and highly purified IFN preparation which has been unavailable with prior art methods which have been costly and provided a poor yield of IFN, limiting the therapeutic potential of IFN.

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L31 ANSWER 2 OF 22 MEDLINE DUPLICATE 1

2000143300 Document Number: 20143300. New approach for **separating** Bacillus subtilis metalloprotease and alpha-amylase by affinity chromatography and for **purifying** neutral protease by hydrophobic chromatography. Lauer I; Bonnewitz B; Meunier A; Beverini M. (Centre de Recherche TEPRAL, Branche Boissons du Groupe Danone, Strasbourg, France.. ilauer@tepral.fr) . JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND APPLICATIONS, (2000 Jan 14) 737 (1-2) 277-84. Journal code: CXN. ISSN: 1387-2273. Pub. country: Netherlands. Language: English.

AB Proteases are commonly used in the biscuit and cracker industry as processing aids. They cause moderate hydrolysis of gluten proteins and improve dough rheology to better control product texture and crunchiness. Commercial bacterial proteases are derived from Bacillus fermentation broth. As filtration and ultrafiltration are carried out as the only recovery steps, these preparations contain also alpha-amylase and beta-glucanase as the main side activities. The aim of this study is to **purify** and characterize the Bacillus subtilis metalloprotease from a commercial preparation, in order to study **separately** the impact of the protease activity with regards to its functionality on biscuit properties. **Purification** was achieved by means of affinity chromatography on Cibacron Blue and HIC as a polishing step. Affinity appeared to be the most appropriate matrix for large scale **purification** while **ion exchange** chromatography was inefficient in terms of recovery yields. The crude product was first loaded on a Hi Trap Blue column (34 microm, Pharmacia Biotech); elution was carried out with a gradient of **NaCl** in the presence of 1 mM ZnCl2. This step was only efficient in the presence of Zn cations, because this salt promoted both protease stabilization resulting in high recovery yields and also complexation of amylase units into **dimers** resulting in amylase retention on the column and a better **separation** of the 3 activities. Beta-glucanase was mostly non retained on the column and a part was coeluted with the protease. This protease fraction was then loaded on a Resource Phe column (15 microm, Pharmacia Biotech) in a last step of polishing. Elution was carried out with a **linear gradient** of 100-0% ammonium sulfate 1.3 M; protease was eluted at the beginning of the gradient and well **separated** from amylase and glucanase trace impurities. The

homogeneity of the **purified** protease was confirmed by SDS-PAGE, which showed that its MW was about 38. pH and temperature optima were also determined on the fraction.

L31 ANSWER 3 OF 22 MEDLINE DUPLICATE 2

2000203947 Document Number: 20203947. Angiotensin converting-like enzymes from urine of untreated renovascular hypertensive and normal patients: **purification** and characterization. Costa R H; Casarini D E; Plavnik F L; Marson O; Alves K B. (Department of Biochemistry, Universidade Federal de Sao Paulo-Escola Paulista de Medicina, SP, Brazil.) IMMUNOPHARMACOLOGY, (2000 Mar) 46 (3) 237-46. Journal code: GY3. ISSN: 0162-3109. Pub. country: Netherlands. Language: English.

AB Angiotensin converting-like enzymes (ACE) were isolated from urine of normal (P0N, P1N and P2N) and untreated renovascular hypertensive (P0, P1 and P2) patients. The urine were submitted to **ion exchange** chromatography. Enzymes P0 and P0N were eluted with the equilibrium buffer (0.02 M Tris-HCl, pH 7.0), while P1, P1N, P2 and P2N with ionic strength **linear gradient** of 0.02-0.5 M Tris-HCl, pH 7.0 in 0.7 mS and P2 and P2N in 1.2 mS conductance. The active fractions were submitted to gel filtration in Sephadex G-150, equilibrated and performed with 0.05 M Tris-HCl/0.15 M **NaCl** buffer, pH 8.0. All enzymes were homogeneous when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (molecular mass: P0, P2 and P2N about 60 kDa; P1, 95 kDa and P21N 170 kDa). The enzymes were recognized by Y1 polyclonal **antibody** raised against human renal ACE. The K(M) values were in millimolar order for hippuryl-L-His-Leu (HHL) while for benzyloxycarbonyl-Phe-L-His-Leu (ZFHL) they were in 10(-4) M order. The enzymes were able to hydrolyze angiotensin I (AI) (P0 and P0N about 25%, P1 and P1N about 70%, P2 100% and P2N 66%) and bradykinin (BK) (P0N 22%, P1N 81%, P2N 62%, P0 and P1 50% and P2 35%), and their activities were inhibited by captopril.

L31 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2001 ACS

1998:699847 Document No. 130:80601 Rapid **separation** of bovine whey proteins by membrane convective liquid chromatography, perfusion chromatography, continuous bed chromatography, and capillary electrophoresis. Girardet, Jean-Michel; Saulnier, Franck; Linden, Guy; Humbert, Gerard (Laboratoire des biosciences de l'aliment, unite associee a l'Inra, Faculte des sciences, universite Henri-Poincare Nancy I, Vandoeuvre-les-Nancy, 54506, Fr.). Lait, 78(4), 391-400 (English) 1998. CODEN: LAITAG. ISSN: 0023-7302. Publisher: Editions Scientifiques et Medicales Elsevier.

AB Membrane convective liq. chromatog. is a technique based on porous cellulose membranes designed for the **sepn.** of biomols. in few minutes at high flow-rates and low back-pressures. Bovine whey proteins are **sepd.** in less than 10 min, at pH 8.5, with a flow-rate of 5.6 mL/min and with a 0-0.2 mol/L **NaCl linear gradient**. Three other rapid methods are also proposed. With the **ion-exchange** perfusion liq. chromatog. based on beads with large pores and with the continuous bed chromatog. based on a polymer matrix, **sepns.** are achieved in only 10 min. Capillary zone electrophoresis using an untreated fused-silica capillary allows the

sepn. of whey proteins in a single run of 8 min without the presence of polymeric additives. These rapid methods are suitable in the quality control of wheys and could be applied in the dairy industry or in research.

L31 ANSWER 5 OF 22 MEDLINE DUPLICATE 3
1998110562 Document Number: 98110562. Characterization of progesterone-binding moieties in the little skate Raja erinacea. Paolucci M; Callard I P. (Department of Biology, Boston University, Massachusetts 02215, USA.) GENERAL AND COMPARATIVE ENDOCRINOLOGY, (1998 Jan) 109 (1) 106-18. Journal code: FL9. ISSN: 0016-6480. Pub. country: United States. Language: English.

AB In this study we report evidence of a [3H]progesterone-binding moiety in the liver and oviduct of the little skate Raja erinacea. It is characterized by high affinity, low capacity and DNA-cellulose-binding activity. Furthermore Western blot analysis revealed that monoclonal **antibodies** against the chicken progesterone receptor (PR) subunits A and B cross-reacted with a 110-kDa band in the liver and a 80-kDa band in the oviduct. When analyzed by DEAE-Sepharose **ion-exchange** column chromatography, [3H]progesterone-binding molecules resolved into two peaks, one nonadherent and one adherent to the column. The liver adherent peak eluted in a **linear gradient** at a **NaCl** concentration of about 0.07 M and resolved on Western blot as a single band of a 110 kDa. The oviduct adherent peak eluted at about 0.14 M **NaCl** and resolved on Western blot as a single band of 80 kDa. Competition studies showed that the progesterone-binding moiety in the cytosol was specific for progesterone. On the contrary, the nuclear component is not specific for progesterone; it also binds testosterone and estradiol 17 beta in the oviduct, and progesterone, testosterone, dihydrotestosterone, estradiol 17 beta, mibolerone, and R5020 in the liver. The [3H]progesterone-binding activity was monitored in both liver and oviduct of females in different reproductive stages. Females were **separated** into three groups; laying, nonlaying, and immature. [3H]Progesterone-binding activity levels were higher in the liver of immature than of nonlaying skates, and it was undetectable in laying skates. [3H]Progesterone binding was higher in the oviduct of laying and nonlaying skates than of immature skates. This PR-binding moiety has many characteristics of a true receptor: high affinity, low capacity, binds to DNA, and cross-reacts with **antibodies** against chicken PR. However, while the cytosolic form of this progesterone-binding component was quite specific for P, nuclear extracted material was nonspecific. If these progesterone-binding components are homologous with the PR A and PR B forms of other vertebrates, as we believe, it is clear that there are species differences that probably relate to phylogenetic level and physiology of the organism.

L31 ANSWER 6 OF 22 CAPLUS COPYRIGHT 2001 ACS
1997:449533 Document No. 127:66101 Preparation of (2'-5').alpha.-oligonucleotides as antiviral agents. Sawai, Hiroaki; Ito, Takeshi; Shinozuka, Kazuo (Yamasa Shoyu Co., Ltd., Japan). Jpn. Kokai Tokkyo Koho JP 09124688 A2 19970513 Heisei, 5 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1995-308109 19951101.

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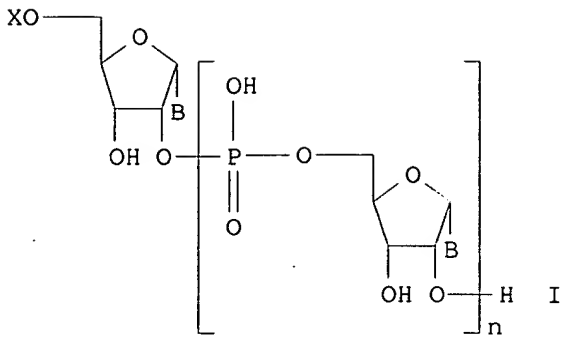

AB The title .alpha.-oligonucleotides (I; B = nucleic acid base; X = H, PO3H2; n = 5-15), which possess biol. activities similar to those of naturally occurring (2'-5')oligoadenylic acid (2-5A) and are less labile to in vivo enzymic cleavage than 2-5A and are expected to be developed as drugs such as antiviral agents (no data), are prepd. in shorter steps by **oligomerization** of .alpha.-nucleoside-5'-phosphoimidazolide in the presence of uranyl ion in an aq. soln. .alpha.-Adenosine (2.0 mmol) (prepn. from 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose and N-benzoyladenine given), 2.4 mmol POCl3, and 10 mL tri-Et phosphate were placed in a flask and allowed to react for 4.5 h in an ice bath and the reaction mixt. was added dropwise to Et2O and the formed ppt. was collected by filtration and dissolved in H2O and passed through a column of activated column which was washed with H2O to remove inorg. H2PO4 and eluted with a 1:20:20 soln. of pyridine/EtOH/H2O. The eluted liq. was concd. and **purified** by an **anion exchange** column using a DEAE-Sephadex A25 eluting the column with a **linear gradient** aq. soln. of 0-0.5 M triethylammonium bicarbonate to give 47% .alpha.-adenylic acid. .alpha.-Adenylic acid (0.82 mmol) was condensed with imidazole 6.4, Ph3P 1.9, dipyridyl disulfide 1.9 mmol, 1.0 mL Et3N, and 0.5 mL trioctylamine in 20 mL DMF at room temp. for 2.5 h to give 63% .alpha.-adenosine-5'-phosphoimidazolide **sodium salt**. The latter compd. (0.225 mmol) was stirred in the presence of 0.009 mmol uranyl nitrate in N-ethylmorpholine buffer at 24.degree. for 4 days to give, after chromatog. **sepn**. using a DEAE-Sephadex A25 eluting the column with a **linear gradient** aq. soln. of 0-1.0 M triethylammonium bicarbonate, **oligomers**, i.e. dimer 26.3, trimer 12.2, tetramer 14.4, pentamer 9.5, hexamer 4.0, and heptamer 1.9%. Each **oligomer** was treated with nuclease P1 decompg. 3'-5' phosphate bonds to give monomer .alpha.-adenylic acid, dimer, trimer, and tetramer contg. 2'-5' phosphate bonds, revealing that long chain **oligomers** mainly contained 3'-5' phosphate bonds. After **purifn**. by preparative HPLC using ODS silica gel column, the yields of (2'-5').alpha.-adenylic acid dimer (.alpha.-pA2'pA) and trimer (.alpha.-pA2'pA2'pA) were 6.4 and 1.4%, resp.

L31 ANSWER 7 OF 22 MEDLINE DUPLICATE 4
1998051100 Document Number: 98051100. Quantitative comparison on the

refinement of horse antivenom by salt fractionation and **ion-exchange** chromatography. Saetang T; Treamwattana N; Suttijitpaisal P; Ratanabanangkoon K. (Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand.) JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND APPLICATIONS, (1997 Oct 24) 700 (1-2) 233-9. Journal code: CXN. Pub. country: Netherlands. Language: English.

AB A quantitative comparison was made on the fractionation of pepsin-digested horse antivenoms by ammonium sulfate (AS) fractional precipitation and **ion-exchange** chromatography on Q-Sepharose. In the precipitation process, pepsin digested horse anti-Naja kaouthia serum was precipitated by 30% saturated AS followed by 50% saturated AS. The recovery of **antibody** activity [as measured by an enzyme-linked immunosorbent assay (ELISA) against the cobra postsynaptic neurotoxin 3] from the 30-50% saturated AS precipitate was 53% with a 1.93-fold **purification**. For the chromatographic process, the behavior of the horse antitoxin **antibody** and its F(**ab**')2 fragments was first studied. The pepsin digested horse serum was then desalted on a Bio-gel P-2 column followed by chromatography on Q-Sepharose using a **linear gradient** (20 mM Tris-HCl, pH 8.0 containing 0.0 to 0.5 M **NaCl**) A peak containing primarily the F(**ab**')2 **antibody** could be obtained. This peak constituted 73% of the total antivenom activity with 2.08-fold **purification**. The total recovery of **antibody** activity by the chromatographic process was 90%. The yield of **antibody** activity was about 2-fold higher than that reported previously with other fractionation procedures. The implications of these results for the refining of horse therapeutic antivenoms are discussed.

L31 ANSWER 8 OF 22 CAPLUS COPYRIGHT 2001 ACS

1995:1003937 Document No. 124:53063 Luminescence immunoassay for allergen using alkaline phosphatase-conjugated **IgE antibody** with flow injection system. Lim, Tae-kyu; Nakamura, Noriyuki; Matsunaga, Tadashi (Dep. Biotechnol., Tokyo Univ. Agriculture and Technol., Tokyo, 184, Japan). Denki Kagaku oyobi Kogyo Butsuri Kagaku, 63(12), 1154-9 (English) 1995. CODEN: DKOKAZ. ISSN: 0366-9297.

AB An allergen detection system was developed based on a luminescence immunoassay with flow injection. Allergen and alk. phosphatase-conjugated **IgE antibody** soln. were mixed, incubated, and analyzed using a **cation exchange** column with a **linear gradient** of **sodium chloride**. The allergen-**antibody** complex was **sepd.** from free alk. phosphatase-conjugated **antibody**. A linear allergen dose response curve was obtained between allergen concn. and the relative luminescence intensity. This simple, rapid, and convenient immunoassay method can detect allergen continuously.

L31 ANSWER 9 OF 22 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1994-206441 [25] WPIDS

AB JP 06145197 A UPAB: 19940810

Isolation of human transforming growth factor (TGF)-beta from biological fluids, involves (1) adjusting the biological fluid at pH 1-3 to convert TGF-beta into an active form, and (2) passing the activated TGF-beta through a **cation exchange** resin (specifically: Fractogel EMD-SO3--650; Merck) bound with SO3(-)-contg. acrylamide

oligomer. If necessary, the biological fluid may preliminarily be applied to gel filtration, and alternatively the fraction eluted from the **cation exchange** resin may further be applied to reverse phase chromatography.

The biological fluid is TGF-beta-contg. cells or tissue extracts including serum, platelet, and cells of pancreas, liver, kidney, placenta or bone. The pH control of the fluid may be made with MeCOOH or HCl and the fluid is allowed to stand over several hrs. or overnight. To remove low mol. contaminant, the fluid may preliminarily be applied to gel filtration using a bridged dextran, agarose, polyacrylamide, partic. Sephadex G-50 (Pharmacia).

USE/ADVANTAGE - Method for highly **purifying** human TGF-beta from biological fluids in high yield.

In an example, human platelet (180 U; 1 U = amt. of platelet isolated from 400 ml blood) was kept in a mixt. of 120 mM HCl, 0.66 mM phenyl-methylsulphonyl fluoride and 80% MeOH with stirring overnight, and the mixt. centrifuged at 10,000 rpm for 30 min. The supernatant was adjusted at pH 3 with 28% ammonia and condensed to the initial vol. (before extraction). The condensate was applied to a column of Sephadex G-50 (9x80 cm) preliminarily equilibrated with 0.1M MeCOOH at a flow rate of 2 ml/min. and eluted with 0.1M MeCOOH to collect every 30 ml fractions.

The active fractions (confirmed by electrophoresis) were applied to a column of Fractogel EMD-SO3(-)-650 equilibrated with 1M MeCOOH at a rate of 1 ml/min., which was washed with 0.1M MeCOOH, 0.1M MeCOOH contg. 2M **NaCl**, and 50 mM ammonia, successively, and eluted with 0-500M **NaCl** (**linear gradient**; 100 min.) in presence of 50 mM ammonia. The active fractions were applied to a column of Vydac C18 (0.46 x 28 cm, 4.0 ml) equilibrated with 0.1% trifluoroacetic acid at a rate of 1 ml/ml and eluted with 0 to 60% MeCN (gradient; 120 min.) to give 500 micro g TGF-beta.

Dwg.0/3

L31 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5

1994:674981 Document No. 121:274981 Biochemical and immunological characterization of .alpha.-amylase isoenzymes of Araucaria araucana.

Acevedo, Elba; Cardemil, Liliana (Fac. Ciencias, Univ. de Chile, Santiago, Chile). Physiol. Plant., 92(1), 149-59 (English) 1994. CODEN: PHPLAI. ISSN: 0031-9317.

AB Seven .alpha.-amylase isoenzymes present in quiescent seeds of the South American conifer Araucaria araucana were **purified** by affinity chromatog. and partially characterized. The mol. masses of these isoenzymes were 45.7, 47.0, 50.2, 51.2, 52.0, 53.5 and 55.2 kDa. The two main isoforms were **sepd**. from each other and from the rest of the isoenzymes by **anion-exchange** chromatog. using a **linear gradient** of 0 to 0.6M **NaCl** and slightly different CaCl2 concns. All isoenzymes bands stained with periodic acid/dansylhydrazine, suggesting that they are glycoproteins. Electroblotting of the isoenzymes onto polyvinylidene difluoride membranes allowed detn. of the amino acid compn. and NH2-terminal sequence of the 53.5-, 50.2- and 47.0-kDa isoenzymes. Amino acid compositional anal. demonstrated that these enzymes are rich in glycine, aspartic acid/asparagine, alanine, serine, proline and glutamic acid/glutamine.

The NH2-terminal sequences of the three isoenzymes are identical. Comparison of the amino acid compns. and the NH2-terminal sequence of these isoenzymes with the cereal and Vigna radiata .alpha.-amylase demonstrated that there is no relation between them. However, polyclonal **antibodies** generated against barley .alpha.-amylase cross-reacted with all the A. araucana .alpha.-amylases. Peptide mapping anal. of the isoenzymes using cyanogen bromide suggests that there are genetic differences between them.

L31 ANSWER 11 OF 22 MEDLINE DUPLICATE 6
93246910 Document Number: 93246910. Evaluation of **cation exchange** chromatography for the isolation of M glycoprotein from histoplasmin. Zancope-Oliveira R M; Bragg S L; Hurst S F; Peralta J M; Reiss E. (Laboratorio de Micologia, Hospital Evandro Chagas, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil..) JOURNAL OF MEDICAL AND VETERINARY
MYCOLOGY, (1993) 31 (1) 29-41. Journal code: JMD. ISSN: 0268-1218. Pub. country: ENGLAND: United Kingdom. Language: English.
AB **Cation exchange** chromatography was evaluated to **purify** the M antigen from histoplasmin (HMIN). Two H and M antigen-containing fractions, soluble (S) and precipitate (PP), resulted from the initial 0.025 M, pH 3.5 citrate buffer dialysis step. The PP fraction contained 62% of the M antigen activity and was resolubilized. Both fractions were chromatographed on CM Sepharose CL-6B. Polysaccharide C antigen was abundant in the S fraction and most of it did not bind to CM
Sepharose. M antigen-enriched fractions were eluted with 0.5 M **NaCl**. Re-chromatography of the relevant S fraction (S-II) and PP fraction (PP-II) by **linear gradient** fast protein liquid chromatography (FPLC) removed protein and C impurities. M antigen **purified** by FPLC from the PP-II fraction was depleted of other antigens when Western blots were probed with anti-M, anti-H and anti-C monoclonal **antibodies** (Mabs). M antigen was identified as a 94 kDa glycoprotein containing a specific-protein epitope and an epitope that
reacted with a Mab against the polysaccharide C antigen. M antigen can be **purified** from HMIN by tandem **cation exchange** chromatography of the precipitable fraction on an open CM Sepharose CL-6B column followed by **linear gradient** FPLC.

L31 ANSWER 12 OF 22 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1991-087405 [12] WPIDS
AB WO 9102977 A UPAB: 19930928
The following are claimed: (A) a pure heparanase formulation having a purity of at least 1000-fold over a crude cell extract of heparanase; (B) a pure heparanase obtainable by (a) contacting a heparanase contg. cell extract with a **cation exchange** resin and obtaining a first active eluate, e.g. by elution with a **linear gradient** of 0.01-1M **NaCl**, (b) contacting the first eluate with an affinity **purification** absorbent and recovering a second active eluate, e.g. by elution with 0.2M'alpha- methyl-mannoside, and (c) recovering a pure heparanase from the second eluate; the affinity absorbent may contain heparin or may be a lectin affinity absorbent.
(C) a method of diagnosing or detecting a heparanasee-related disease
state in an individual which comprises contacting a body fluid of the

individual with a detectably labelled **antibody** having specificity for heparanase and observing the presence or absence of a reaction between the labelled **antibody** and heparanase; (D) a monoclonal or polyclonal **antibody** having specificity for heparanase; (E) a method of treatment of wounds which comprises applying to the wound a **purified** heparanase.

USE/ADVANTAGE - The heparanase is useful in situations in which angiogenesis and/or the growth of fibroblasts is desired, e.g. in the healing of wounds such as cuts, burns, ulcers and ischaemic tissues. It can also be used in promoting bone healing by initiating neovascularisation in a fractured area..The heparanase **antibodies** can be used for detection, quantitation and localisation of heparanase for diagnostic purposes, e.g. detection of metastatic cancer.

0/8

ABEQ EP 487627 A UPAB: 19930928

The following are claimed: (A) a pure heparinase formulation having a purity of at least 1000-fold over a crude cell extract of heparinase; (B) a pure heparinase obtainable by: (a) contacting a heparinase contg. cell extract with a **cation exchange** resin and obtaining a first active eluate, e.g. by elution with a **linear gradient** of 0.01-1M **NaCl**; (b) contacting the first eluate with an affinity **purificn.** absorbent and recovering a second active eluate, e.g. by elution with 0.2M'alpha- methyl-mannoside; and (c) recovering a pure heparinase from the second eluate, the affinity absorbent may contain heparin or may be a lectin affinity absorbent; (C) a method of diagnosing or detecting a heparinase-related disease state in an individual which comprises contacting a body fluid of the individual with a detectably labelled **antibody** having specificity for heparinase and observing the presence or absence of a reaction between the labelled **antibody** and heparinase; (D) monoclonal or polyclonal **antibody** having specificity for heparinase; and (E) a method of treatment of wounds which comprises applying to the wound a **purified** heparinase.

USE/ADVANTAGE - The heparinase is useful in situations in which angiogenesis and/or the growth of fibroblasts is desired, e.g. in the healing of wound such as cuts, burns, ulcers and ischaemic tissues. It can also be used in promoting bone healing by initiating neovascularisation in a fractured area. The heparinase **antibodies** can be used for detection, quantitation and localisation of heparinase for diagnostic purposes, e.g. detection of metastatic cancer.

ABEQ US 5362641 A UPAB: 19941223

Heporanase formulation from the Sk-Hep-1 cell line has hydrolytic activity for heparan sulfate but not heparin of at least 1000-fold greater than that of a centrifuged dialysed cell homogenate prepd. from the cell line.

USE - For stimulating wand healing.

Dwg.0/12

L31 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7

1991:513143 Document No. 115:113143 Isolation of lactoperoxidase and lactoferrins from bovine milk acid whey by carboxymethyl **cation**

exchange chromatography. Yoshida, Shigeru; Ye, Xiuyun (Dep. Appl. Biol. Sci., Hiroshima Univ., Japan). J. Dairy Sci., 74(5), 1439-44 (English) 1991. CODEN: JDSCAE. ISSN: 0022-0302.

AB Lactoperoxidase and lactoferrin were isolated by carboxymethyl **cation-exchange** chromatog. using 0.05M phosphate buffer (pH 7.7) and a **linear gradient** of **NaCl** from 0 to 0.55M. Carboxymethyl-Toyopearl adsorbed only lactoperoxidase and lactoferrin from the **albumin** fraction of bovine milk acid whey. Lactoperoxidase was released between 0.10 and 0.15M **NaCl** with a recovery of 91.4%. Lactoferrin was released between 0.4 and 0.55M **NaCl** and was **sepd.** into lactoferrin-a and lactoferrin-b. Yields were 41 mg of lactoperoxidase, 21 mg of lactoferrin-a, and 67 mg of lactoferrin-b as protein from 1 L of acid whey. Several minor peaks eluted between 0 and 0.55M **NaCl**, and a strong muddy peak was obsd. during the regenerating phase using 0.2N NaOH. The latter peak was estd. as the Ig.

L31 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8

1991:488017 Document No. 115:88017 Examining the possible isolation of factor IX complex by **ion-exchange** column chromatography. Azigirova, M. A.; Vyazova, E. P.; Shaprova, N. N.; Kheilomskii, A. B.; Zeinalov, A. M. (Vses. Gematol. Nauchn. Tsentr., Moscow, USSR). Khim.-Farm. Zh., 25(6), 59-60 (Russian) 1991. CODEN: KHFZAN. ISSN: 0023-1134.

AB The study was undertaken to examine if the TSK gel, DEAE-Toyopearl (Japan) might be used in the chromatog. **sepn.** of plasma supernatant to prep. a blood coagulation factor IX conc. (FIX C). When applying a **linear gradient** ionic from 0.15 to 2.0M **NaCl**, **albumin**-free FIX C was obtained of a specific activity 686.7 units/g protein, as calcd. for Factor IX, which was nearly 50 times as high as that for the starting sample of a plasma cryosupernatant. Stepwise elution allows one to **purify** FIX C of factor VII. Thus, plasma cyrosupernatant **sepn.** with a DEAE-Toyopearl **anion exchanger** isolates FIX C from the major plasma proteins, suggesting that the sorbent might be promising for preparative isolation of FIX C.

L31 ANSWER 15 OF 22 MEDLINE DUPLICATE 9

91097781 Document Number: 91097781. A protocol for the **purification** of bovine **serum albumin** free of deoxyribonuclease activity. Trujillo L E; Castellanos L; Garcia O; Herrera L. (Center for Genetic Engineering and Biotechnology, Havana, Cuba..) BIOTECHNIQUES, (1990 Nov) 9 (5) 620-2. Journal code: AN3. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB Bovine **serum albumin**, free of deoxyribonuclease activity, was obtained in our laboratory using **ion-exchange** chromatography followed by acetylation. Chromatography on four different resins (DEAE-52, P-11, hydroxylapatite and Q Sepharose fast-flow) was examined. Fractions from Q Sepharose chromatography, eluted with a **linear gradient** 0-1.0 M **NaCl** and subsequently acetylated, proved to be the most effective method for obtaining deoxyribonuclease-free bovine **serum albumin**.

L31 ANSWER 16 OF 22 CAPLUS COPYRIGHT 2001 ACS
1990:73388 Document No. 112:73388 Chromatographic stationary phases with affinity, **ion-exchange**, or hydrophobic surfactants, their preparation, and their use. Carbonell, Ruben G.; Kilpatrick, Peter K.; Torres, Juan Luis; Guzman, Roberto (North Carolina State University, USA). PCT Int. Appl. WO 8904203 A1 19890518, 54 pp. DESIGNATED STATES: W: JP, KR; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1988-US4045 19881110. PRIORITY: US 1987-119020 19871110; US 1988-268811 19881108.

AB Chromatog. apps. (i.e. columns) incorporating an improved means of connecting a ligand to a hydrophobic solid support, e.g. hydrophobic silica particles or hydrophobic polymers, are provided, as are compns. and methods for their prepn. Bound to the solid support are surfactants comprising (1) a polar group, (2) a hydrophobic functional group substituted on the polar group, and (3) a chromatog. functional group substituted on the polar group. Preferable polar groups are polyalkoxy groups. The chromatog. functional group is (1) a ligand for affinity chromatog., (2) an iogenic group for **ion-exchange** chromatog., or (3) a hydrophobic group for hydrophobic chromatog. Covering surfactants are preferably adsorbed to the solid support to reduce nonspecific binding thereto. The invention provides a means for easily reversibly binding chromatog. functional groups to a solid support.

Capacity of the solid support for the chromatog. functional groups is increased. Pyridinium, a specific cholinesterase inhibitor, was coupled to octaethylene glycol mono-n-hexadecyl ether (C16E8) by tresylation of the surfactant followed by nucleophilic substitution with the inhibitor; the product was **purified** in 71.9% yield by preparative reversed-phase HPLC. To a com. precolumn (2 cm length, 2 mm inside diam.) packed with 0.021 g of Davisil octadecyl-bonded silica (400 .ANG. pore size, 30-40 .mu.m particle size) was applied a 10 .mu.M soln. of the C16E8-pyridinium until absorbance at 259 nm was const. The specific adsorption of the affinity surfactant to the reversed-phase material was 0.302 .mu.mol/mg packing. The column was equilibrated with 0.05M Tris-HCl buffer (pH 8.0) contg. 0.1M **NaCl** at a flow rate of 1.0 mL/min. To the column was applied 100 .mu.L of a mixt. of horse serum cholinesterase and bovine **serum albumin** (0.70 mg total protein/mL, 22.1 units enzyme activity/mg). All of the cholinesterase activity was retained; >90% of the cholinesterase activity was recovered by application of a sharp two-minute **linear gradient** to 0.05M Tris-HCl (pH 9.0) contg. 1.0M **NaCl**. The specific activity of the eluate was 250 units/mg, corresponding to an 11-fold **purifn**. The affinity surfactant is easily removed from the column by washing the column with 6:4 MeOH/Me2CHOH.

L31 ANSWER 17 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
1990:70246 Document No.: BA89:38072. **PURIFICATION** OF MOUSE MONOCLONAL IMMUNOGLOBULIN M BY **ION-EXCHANGE** LIQUID CHROMATOGRAPHY. ROTHMAN S W; GENTRY M K; GAWNE R D; DOBEK A S; OGERT R; STONE M J; STRICKLER M P. WALTER REED ARMY INST. RES., WASHINGTON, D.C. 20307, USA.. J LIQ CHROMATOGR, (1989) 12 (10), 1935-1948. CODEN: JLCHD8. ISSN: 0148-3919. Language: English.

AB Immunoglobulin M (IgM) **antibodies** have been difficult to **purify**. By eluting with a gradient of increasing salt and decreasing pH, we succeeded in highly **purifying** mouse monoclonal IgM **antibodies** in one step on **ion-exchange** high performance liquid chromatography (HPLC). Two of the **antibodies** were specific for Clostridium difficile toxin A and toxin B and three were raised against a synthetic peptide mimicking the active site peptide of Torpedo californica 11s acetylcholinesterase. the ascitic fluids were diluted 1:10 in start buffer (20 mM Tris HCl, pH 8.5) with enough **NaCl** to allow solubilization of the IgM, and injected onto a diethylaminoethyl (DEAE) 5PW column 7.5 mm .times. 7.5 cm, equilbrated with start buffer. Elution was as follows: (1) 10 min **linear gradient** to 50% start buffer, 50% limit buffer (20 mM Tris HCl, 0.3 M **NaCl**, pH 7.0); (2) 10 min, no change; (3) 10 min **linear gradient** to 100% limit buffer; (4) 20 min, no change. Flow rate was 1 ml/min. IgM eluted at 30 min, at a conductance of 20 mmho and a pH of 7.6. IgM was clearly **separated** from transferrin (12 min), IgG (14, 15 min) an **albumin** (19 min). Confirmation that the 30 min peaks contained IgM was obtained using a solid-phase enzyme-linked immunosorbent assay (ELISA) with goat anti-mouse IgM labelled with alkaline phosphatase as the detecting **antibody**. Immunoreactivity of the IgM-containing peaks ith C. difficile toxin A was confirmed by ELISA. **Purified antibody** was successfully coupled to alkaline phosphatase and was used to detect C. difficle toxin A in an ELISA.

L31 ANSWER 18 OF 22 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 10
1990:156812 Document No. 112:156812 Effects of chromatographic parameters on the fractionation of whey proteins by **anion-exchange** FPLC. Girardet, J. M.; Paquet, D.; Linden, G. (Fac. Sci., Univ. Nancy 1, Fr.). Milchwissenschaft, 44(11), 692-6 (English) 1989. CODEN: MILCAD. ISSN: 0026-3788.

AB The influence of several chromatog. parameters, e.g. pH, flow rate, salt ions, ionic strength gradient, on the resoln. of whey proteins **sepd.** by **anion exchange** FPLC (fast protein liq. chromatog.; Mono Q column) was studied. A linear ionic strength gradient of 0-0.35 M **NaCl** in 20 mM tris-HCl, pH 7, was used to **sep.** bovine **serum albumin** from other whey proteins. .alpha.-Lactalbumin was obtained rapidly by a stepwise gradient with the same buffer system. For **sepg.** .beta.-lactoglobulins B and A from each other, a **linear gradient** at pH 7 was used. However, a stepwise gradient at pH 8 was preferable. With a linear or stepwise gradient of 0.05-0.7 M sodium acetate, pH 6.3, both genetic variants were well resolved. At this pH value, Igs were not retained on the column as much as at pH 8 and were practically eluted in the void vol. .alpha.-Lactalbumin and bovine **serum albumin** were eluted simultaneously.

L31 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2001 ACS
1988:566605 Document No. 109:166605 **Separation** of oligonucleotides

by high-performance **ion-exchange** chromatography on a non-porous **ion exchanger**. Kato, Yoshio; Kitamura, Takashi; Mitsui, Akane; Yamasaki, Yosuke; Hashimoto, Tsutomu; Murotsu, Tomoaki; Fukushige, Shinichi; Matsubara, Kenichi (Cent. Res. Lab., Tosoh Corp., Shinnanyo, 746, Japan). J. Chromatogr., 447(1), 212-20 (English) 1988. CODEN: JOCRAM. ISSN: 0021-9673.

AB The column was TSK gel DEA-NPR (35 mm .times. 4.6 mm, inner diam.) packed with nonporous spherical hydrophilic resins of 2.5 .mu.m in diam. whose surfaces are chem. bonded with diethylaminoethyl groups. Elution was usually performed with a **linear gradient** of **NaCl** in 20 mM Tris-HCl buffer (pH 9.0) or 1,3-diaminopropane-HCl buffer (pH 10.5) at a flow-rate of 1.5 mL/min and 25.degree.. In some **sepns.**, however, the conditions were varied to study their effects. Oligonucleotides were **sepd.** rapidly with very high resoln. and recovery, mainly based on the chain length at pH 8.5-9.5. The **sepn.** of oligonucleotides mainly according to the base compn. is also possible by using an eluent of high pH around 10.5. Chromatog. conditions such as the eluent pH, type of salt, addn. of org. solvent to the eluent, temp., gradient steepness, flow-rate and column length influence the retention, selectivity, recovery, resoln., etc.

L31 ANSWER 20 OF 22 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 11

1987:571985 Document No. 107:171985 Isolation of .alpha.2-globulin associated with Crohn's disease. Chekhonin, V. P.; Khalif, I. L.; Kirkin, B. V.; Ovchinnikov, A. V. (Scientific-Research Institute of Proctology, USSR). U.S.S.R. SU 1318915 A1 19870623 From: Otkrytiya, Izobret. 1987, (23), 157-8. (Russian). CODEN: URXXAF. APPLICATION: SU 1985-3973473 19851118.

AB .alpha.2-Globulin assocd. with Crohn's disease is isolated from affected tissue of the gastrointestinal tract by extn. of homogenated tissue with detergents in pH 8.6 buffer, salting-out, and chromatog. on an **anion exchange** resin eluted with 0.25-0.5 M **linear gradient NaCl**. The fractions eluting with 0.38-0.46 M **NaCl** were further **sepd.** by isoelec. chromatog. pH 4.8-5.6 and gel filtration. . .alpha.2-Globulin was **purified** from an 85,000 .+-. 10 mol. wt. fraction by affinity chromatog. using **antibody** immunosorbent and elution with 0.02 M buffer pH 2.2.

L31 ANSWER 21 OF 22 MEDLINE DUPLICATE 12

86278083 Document Number: 86278083. **Purification** and biologic properties of fibroblast somatomedin. Clemmons D R; Shaw D S. JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Aug 5) 261 (22) 10293-8. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cultured human fibroblasts produce a peptide growth factor that cross-reacts with antisera to human somatomedin-C (Sm-C). To determine the identity of this species and compare its molecular properties to pure Sm-C, 2 liters of conditioned medium derived from human fibroblast monolayers were concentrated (X10) by ultrafiltration. The concentrated conditioned medium was **purified** further by CM-Sephadex **ion-exchange** chromatography. Following elution in 1.0 M **NaCl**, pH 8.0, the active material was **purified** by gel filtration on Sephadex G-150. The active fractions which eluted at Kd 0.45

(Mr estimated at 32,000) were further **purified** by isoelectric focusing. Two peaks of activity electrofocused at pI 5.4 and 7.2, respectively. The pI 5.4 peak contained only binding protein activity. The active fractions from the neutral pool were further **purified** by reverse-phase high pressure liquid chromatography on a C-18 Bondapak with a **linear gradient** of acetonitrile (10-60%). The active single peak which eluted at 55% acetonitrile gave a single band when analyzed by polyacrylamide gel electrophoresis. This material stimulated [3H]thymidine incorporation into human fibroblast DNA with approximately 3.2 times the potency of pure Sm-C but was equipotent in stimulating BALB/c 3T3 fibroblasts. It was degraded by fibroblast cultures at a slower rate compared to Sm-C, although it had a similar affinity for Sm-C-binding protein. We conclude that human fibroblasts produce two peptides that react with anti-Sm-C **antibody** but are chemically distinct from Sm-C. The greater response to fibroblast somatomedin may be due to its affinity for somatomedin-binding protein and slower degradation. These findings may have implications for understanding the regulation of human fibroblast replication.

L31 ANSWER 22 OF 22 MEDLINE DUPLICATE 13
85021433 Document Number: 85021433. **Purification** and characterization of pregastric esterase from calf. Sweet B J; Matthews L C; Richardson T. ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1984 Oct) 234 (1) 144-50. Journal code: 6SK. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB Calf pregastric esterase (PGE) was **purified** from calf gullet tissues. The tissue was excised and lyophilized, and lipid materials were extracted with acetone and n-butanol at -20 degrees C. Proteins were extracted from the delipidated tissue with a buffer containing a chaotropic salt (NaSCN) to solubilize hydrophobically bound protein aggregates. Calf PGE precipitated from the crude extract at pH 5.0. The precipitated, solubilized proteins were subjected to **anion-exchange** chromatography on DEAE-Sephacel, and the enzymatic activity was eluted using a **linear gradient** from 0.10 to 0.50 M **NaCl** at pH 8.0. Fractions with high specific activities were then chromatographed twice using gel filtration on Sephadex G-100. The resultant enzyme was shown to be pure upon discontinuous electrophoresis in 12% polyacrylamide containing 0.1% sodium dodecyl sulfate (SDS-PAGE). From SDS-PAGE gel patterns, a molecular weight of 49,000 was determined. The amino acid composition of the enzyme allowed calculation of an "average hydrophobicity" (Bigelow index) of 1150 cal/residue. This indicates that calf PGE is relatively hydrophobic, being similar to proteins such as alpha-lactalbumin and bovine **serum albumin** in average hydrophobicity.

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'IN' IS NOT A VALID FIELD CODE

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L32             0 FILE MEDLINE
L33             1 FILE CAPLUS
L34             0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L35             0 FILE EMBASE
L36             1 FILE WPIDS

TOTAL FOR ALL FILES
L37             2 ANSALDI D?/AU,IN

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L38             1 DUP REM L37 (1 DUPLICATE REMOVED)

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L38  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2001 ACS          DUPLICATE 1
1999:784126   Document No. 132:20776  Separation of protein monomers from
     aggregates by use of ion-exchange chromatography.  Ansaldi,
     Deborah; Lester, Philip (Genentech, Inc., USA).  PCT Int. Appl. WO
     9962936 A1 19991209, 23 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU,
     AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD,
     GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
     LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE,
     SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY,
     KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE,
     DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,
     TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1999-US11598
     19990524.  PRIORITY: US 1998-87602 19980601.
AB   A method is disclosed for sepg. a polypeptide monomer from a mixt.
     comprising dimers and/or multimers.  The method comprises applying the
     mixt. to either a cation-exchange chromatog. resin or an anion-exchange
     chromatog. resin and eluting the mixt. at a gradient of about 0-1 M of an
     elution salt, wherein the monomer is sepd. from the dimers and/or
     multimers present in the mixt.  Monoclonal antibodies to IgE, IgE, and
     bovine serum albumin samples were sepd. on various ion-exchange resins
     with various buffer and elution salt conditions.


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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
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FULL ESTIMATED COST                                  128.69     130.39

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L9 ANSWER 24 OF 47 MEDLINE
ACCESSION NUMBER: 1998409447 MEDLINE
DOCUMENT NUMBER: 98409447 PubMed ID: 9737871
TITLE: Factors influencing the dimer to monomer transition of an antibody single-chain Fv fragment.
AUTHOR: Arndt K M; Muller K M; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (1998 Sep 15) 37 (37) 12918-26.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981029
Last Updated on STN: 19981029
Entered Medline: 19981020

AB Antibody single-chain Fv (**scFv**) fragments are able to form dimers under certain conditions, and the extent of dimerization appears to depend on linker length, antibody sequence, and external factors. We analyzed the factors influencing dimer-monomer equilibrium as well as the rate of interconversion, using the **scFv** McPC603 as a model system. In this molecule, the stability of the VH-VL interaction can be conveniently varied by adjusting the ionic strength (because of its influence on the hydrophobic effect), by pH (presumably because of the presence of titratable groups in the interface), and by the presence or absence of the antigen phosphorylcholine, which can be rapidly removed due to its very fast off-rate. It was found that the monomer is the thermodynamically stable form with linkers of 15 and 25 amino acids length under all conditions tested (35 &mgr;M or less). The dimer is initially formed in periplasmic expression, presumably by domain swapping, and can be trapped by all factors which stabilize the VH-VL interface, such as the presence of the antigen, high ionic strength, and pH below 7.5. Under all other conditions, it converts to the monomer. Predominantly monomer is obtained during in vitro folding. Monomer is stabilized against dimerization at very high concentrations by the same factors which stabilize the VH-VL interaction. These results should be helpful in producing molecules with defined oligomerization states.

(FILE 'HOME' ENTERED AT 07:03:19 ON 06 OCT 2000)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH, TOXLINE'
ENTERED AT 07:03:46 ON 06 OCT 2000

L1	360027	S MONOMER##
L2	5663429	S POLYPEPTIDE OR PROTEIN OR IMMUNOGLOBULIN
L3	1488922	S CHROMATOGRAPH###
L4	584577	S CATION OR ANION
L5	263	S L1 (30A) L2 (30A) (L3 (10A) L4)
L6	86	DUP REM L5 (177 DUPLICATES REMOVED)
L7	20728	S (CATION OR ANION) (3A) (EXCHANGE CHROMATOGRAPHY)
L8	132	S L1 (30A) L2 (30A) L7
L9	44	DUP REM L8 (88 DUPLICATES REMOVED)
L10	280842	S DIMER## OR MULTIMER##